Neurotoxic Effects of Cypermethrin in Wistar Rats: a Haematological, Biochemical and Histopathological Study

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Cypermethrin is a synthetic pyrethroid that belongs to a group of insecticides with low mammalian toxicity but high insecticidal activity. The present study was undertaken to evaluate subacute toxicity of orally administered cypermethrin in rats based on the haematological, enzymological, and histological brain examination. The study was conducted on 8-week old male Wistar rats and Kral 250 EC (250 g of cypermethrin/l) was orally administered (60, 150, and 300 mg/kg) for 28 consecutive day. Thus, the investigation covered four groups of animals: three experimental groups and one control group, of 20 rats each. Our results demonstrated that experimental groups receiving both 150 and 300 mg/kg cypermethrin treatment led to significant dose–dependent decrease in some haematological parameters [red blood cell (RBC) counts, haematocrite (Ht), thrombocyte, and mean corpuscular haemoglobin (MCH) values] at the end of the experiment. When compared with control animals, there were significant increases between initial body weights and final body weights of cypermethrin treated rats, but there was a significant decrease in brain/body weight ratio of the animals of all treated groups. Furthermore, there was no statistical difference between control group and all experimental groups for brain acetylcholinesterase (AChE) and blood cholinesterase (ChE) enzyme activities, although brain AChE activities were increased in rats treated with a dose of 150 and 300 mg/kg cypermethrin. Histologically, some deformation areas due to ischemia and pyknosis of the cytoplasm of the neurons were observed in brain tissues of rats treated with all doses of cypermethrin.

Key words —— cypermethrin, haematology, brain, histology, rat

INTRODUCTION

Synthetic pyrethroids, a unique group of insecticides having pyrethrin-like structure with better performance characteristics and globally account for over 30% of insecticide use.[1] Pyrethroids are modified derivates of pyrethrins, natural substances obtained from the flowers of *Pyrethrum* species.[2,3] At present, they have broad-spectrum use in agriculture, domestic and veterinary applications due to their high bioefficacy, enhanced stability, and comparatively low mammalian toxicity.[4–6] Pyrethroid insecticides have been divided into types I and II based on behavioral profiles of toxicity. Type I pyrethroids may cause mainly hyperexcitation and fine tremors and type II pyrethroids such as cypermethrin, produce a more complex syndrome including clonic seizures, but the signs of toxicity disappear fairly rapidly and they recover within a week.[7] Cypermethrin [(RS)-cyano-(3-phenoxyphenyl) methyl (1RS)-cis-,trans-3-(2,2-dichloroethenyl)-2.2-dimethylcyclopropane carboxylate] is a synthetic pyrethroid insecticide which is used to kill the insects especially on cotton.

The absorption of cypermethrin from the digestive tract and its excretion takes a quick course. It is well established that cypermethrin, both cis- and trans- isomers are metabolized to phenoxybenzoic acid and cyclopropanecarboxylic acid.[3] In humans, urinary excretion of cypermethrin metabolites was complete 48 hr after the last of five doses of 1.5 mg/kg/day, but studies in rats have shown that cypermethrin is rapidly metabolized by hydroxyla-

tion and cleavage, with over 99% being eliminated within hours. The remaining 1% becomes stored in body fat and this portion is eliminated slowly, with a half-life of 18 days for the cis-isomer and 3, 4 days for the trans-isomer.[8] Interestingly, cypermethrin persists in air and on walls and furniture for about three months after household treatments.[9] In spite of the low toxicity of pyretroids, persistence of these compounds in mammalian tissues may be dangerous.[4] Permanence of cypermethrin and its fatty acid conjugates in adipose tissue, brain and liver was reported in rats.[10,11] However, several authors reviewed pyrethroid neurotixicity, concluding that pyrethroids do not accumulate in the body and their removal from nervous system as well as their excretion are rapid, even after repeated administrations.[12,13]

Some studies have demonstrated that synthetic pyrethroid insecticides are toxic to the mammalian central nervous system in acute intoxication.[14] In particular, it affects axons of the neurons of the peripheral and central nervous system and interacts with the transportation system of sodium ions through the cellular membrane.[7,8] This results a delay in the closing of sodium channel and a prolonged sodium tail current after the membrane repolarization.[15,16] Thus, cypermethrin, a specific calcineurin inhibitor, is acting a neurotoxin for both insects and mammals. Husain *et al*.[17] studied the neurotoxic action of a synthetic pyrethroid, deltamethrin and it was reported that acetylcholinesterase (AChE) activity was markedly increased in the different regions of the brain.

This is a repeated dose 28-day oral toxicity study in rodent (OECD 407) and it has been designed to assess a wide range of toxic end-points and include measurements of multiple biochemical, haematological, pathological and organ weight endpoints.[18] There is no doubt that hematology and clinical chemistry results add necessary perspective for the identification of target organs and may contribute to an understanding of the mechanism of action. These informations are necessary for the risk assessment before the microscopic evaluation commences. The effect of decamethrin, another pyrethroid insecticide, on certain haemato-biochemical characteristics were determined in male rabbits.[19] However, there is limited research investigating the effects of cypermethrin on histology and AChE activitiy of the brain tissue, cholinesterase (ChE) enzyme activity in blood, and some haematological parameters in the literature. The objective of this study was to evaluate subacute toxicity of the orally administered cypermethrin in adult male Wistar rats based on the results of haematological, biochemical, and histopathological investigations. In our study ChE enzyme was choosed the biochemical indicator of neurotoxicity in addition to brain histopathology.

MATERIALS AND METHODS

Chemical Substances —— Commercial formulation of cypermethrin (250 g of cypermethrin/l), Kral 250 EC (Safa Agriculture, Turkey), was used. It was in the form of emulsion and adequate dilutions were done in water in order to reach the above test concentrations. The test concentration of cypermethrin was calculated from the percentage of the active ingredients. The oral LD_{50} for cypermethrin in male and female rats were 187 to 326 and 150 to 500 mg/kg, respectively.[20,21] Therefore, 60 mg/kg (1/5 of LD_{50}) as low dose, 150 mg/kg (two fold of low dose) as medium dose, and 300 mg/kg (two fold of medium dose) as high dose were selected in the study. Solutions were freshly made immediately before usage. All the other reagents used were of analytical reagent grade and obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals and Experimental Design —— The protocol was approved by the Animal Ethical Committee of Ege University Faculty of Medicine. This experiment was conducted on 80 adult male (8-week old) Wistar rats (120–160 g in weight) obtained from Breeding Center of Experimental Animals in Ege University Faculty of Medicine. After 10 days of acclimation, the animals were randomly assigned to either the experimental groups (low dose group, 60 mg/kg; medium dose group, 150 mg/kg; and high dose group, 300 mg/kg) or the control group, each containing 20 rats and housed individually in labelled cages with solid plastic sides and stainless-steel grid tops and floors. Animals of the control group were orally fed daily with a normal diet in standard laboratory chow (10 g/rat/day), while rats in treatment groups were fed with laboratory chow (10 g/rat/day) combined with cypermethrin. In this study, cypermethrin was orally administered for 28 consecutive days as described in OECD guideline 407.[18] They were maintained in controlled laboratory conditions of 12 hr dark/light cycle, 21 ± 1°C temperature and 45–75% humidity. Tap water was also available *ad libitum*. All animals were weighed weekly throughout the study.

Haematological Analysis —— At the end of the

experiment, 10 rats of each group were anaesthetized with ether and blood samples were drawn from the heart of each animal. Two blood samples were taken with or without EDTA. The one with EDTA was used for haematological analysis, and the other for preparation of serum for the biochemical assays. Blood samples with anti-coagulant EDTA were analysed for blood parameters namely red blood cell (RBC) counts, white blood cell (WBC) counts, thrombocytes, haemoglobin (Hb), haematocrite (Ht), differential leucocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) using CELL-DYN 1700 counter (Abbott Hematology Analyser Cell-Dyn 1700, Abbott Laboratories, Abbott Park, Illinois, U.S.A.) in Atatürk Education Hospital in İzmir, Turkey.

Biochemical Assay —— After centrifugation of the blood samples without EDTA at 5000 rpm for 20 min at 4°C, serum was separated and stored at –70°C until the analysis. The animals used in haematological assay were killed by cervical dislocation and the brains of them were dissected out, weighed and stored at –70°C until analysis. The brain tissues (1 g) homogenized with Ultra Turrax homogeniser in 5 ml of 50 mM phosphate buffer (pH 7.4). The particle-free supernatant was obtained by centrifugation at 5000 rpm for 20 min at 4°C and used as enzyme source. The blood ChE and brain AChE activities were determined by the spectrophometric method as previously described by Ellman *et al.*[22] The assay mixture contained 0.259 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 67 mM phosphate buffer, pH 7.4, 0.298 mM acetylthiocholine chloride and 20 μl of 250-fold dilution of the enzyme source in a total volume of 3.02 ml. Reaction was followed at 410 nm for 10 min intervals at 37°C against blank containing acetylthiocholine chloride and phosphate buffer. The extinction coefficient of the product of the chemical reaction, 5-thio-2-nitrobenzoate is 13.61 mM^{-1} cm^{-1}. The protein content was estimated as described by Lowry *et al.*[23]

Histopathologic Examination —— For light microscopic examination, other 10 animals of each group were anaesthetised (0.10 mg/kg Ketalar® + 0.02 mg/kg Rompun®, i.p.) and perfused transcardially with 100 ml heparinised saline followed by 300 ml of 4% para-formaldehyde in 0.1 mol/l phosphate buffer (pH: 7.4). Brains were removed and their weights were recorded and brain/body weight ratios of each animal were calculated. Then, they post-fixed for 24 hr in the same fixative, and

processed for paraffin embedding. After routine processing, paraffin sections of each tissue were cut into 5–6 μm thickness and stained with haematoxylin and eosin (H&E).

Statistical Analysis —— The results of haematological and biochemical analyses, brain weight and brain/body weight ratios were presented as the mean ± SEM. Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of $p \leq 0.05$ were regarded as statistically significant.

RESULTS

Changes in Haematological Parameters

The results of haematological analysis of rats in control and treatment groups are given in Table 1. Normal haematological values for rats were also shown in Table 1.[24,25] In rats treated with 150 and 300 mg/kg cypermethrin, RBC counts, Ht, thrombocyte and MCH values showed a significant dose–dependent decrease. In addition, 300 mg/kg cypermethrin treatment caused a significant decrease in Hb value. Cypermethrin caused a significant dose–dependent decrease in MCV values of all treated groups. There was no statistically significant increase in WBC counts, but a significant increase in the numbers of lymphocyte and monocyte was observed in animals administrated 300 mg/kg cypermethrin when it was compared with the control ($p < 0.05$ and $p < 0.05$, respectively). Pronounced changes were determined in other haematological parameters, but these changes were not dose–dependent and statistically significant.

Body and Brain Weights

In comparison with control animals, there was no significant change in body weight gains of cypermethrin treated rats (Table 2). No significant change was found in the brain weights of rats treated with cypermethrin (Table 2). However, a significant decrease in relative brain weights of the animals treated with cypermethrin in all doses was determined ($p < 0.05$).

Biochemical Findings

Data of total proteins and brain AChE and blood ChE enzyme activities of exposure groups are shown in Figs. 1 and 2. There was no statistically significant change in plasma and brain total proteins and blood ChE and brain AChE enzyme activities be-

Table 1. Results of Haematological Analysis of Rats in Control and Cypermethrin Treatment Groups ($n = 10$)

Parameters	Normal Values[24,25]	Control	60 mg/kg	150 mg/kg	300 mg/kg
RBC ($\times 10^{12}$/l)	6–10	7.48 ± 0.11	7.30 ± 0.45	5.78 ± 0.77*	4.26 ± 0.49*
Hb (g/dl)	11–18	14.4 ± 1.13	15.5 ± 0.40	14.6 ± 1.07	8.1 ± 1.71*
Ht (%)	34–48	43.2 ± 5.23	37.1 ± 2.02	30.5 ± 4.10*	21.0 ± 2.15*
WBC ($\times 10^9$/l)	7–14	6.99 ± 1.08	9.36 ± 2.74	10.43 ± 3.58	16.13 ± 6.07
Thrombocytes ($\times 10^9$/l)	800–1500	817.5 ± 36.5	706.4 ± 71.7	452.0 ± 136.2*	323.0 ± 71.5*
Lymphocyte ($\times 10^9$/l)	3–12	7.49 ± 0.77	10.45 ± 1.93	12.06 ± 2.95	18.20 ± 2.13*
Monocyte ($\times 10^9$/l)	0.0–0.5	0.23 ± 0.15	0.63 ± 0.15	0.56 ± 0.08	0.90 ± 0.11*
Granulocyte ($\times 10^9$/l)	0.0–0.3	0.10 ± 0.05	0.10 ± 0.03	0.16 ± 0.05	0.13 ± 0.02
Lymphocyte (%)	65–84.5	95.5 ± 2.72	94.0 ± 1.31	92.0 ± 2.23	93.1 ± 1.91
Monocyte (%)	0–5	3.07 ± 1.41	5.22 ± 2.02	5.53 ± 1.50	5.64 ± 1.39
Granulocyte (%)	0–4	1.31 ± 0.69	0.57 ± 0.06	2.40 ± 1.64	1.16 ± 0.55
MCV (fl)	50–65	54.5 ± 1.46	52.1 ± 0.50*	51.3 ± 0.43*	49.8 ± 0.98*
MCH (pg)	19–23	18.9 ± 0.70	18.8 ± 0.32	17.0 ± 1.11*	17.1 ± 0.45*
MCHC (g/dl)	32–38	34.5 ± 2.13	34.2 ± 1.19	33.2 ± 2.11	34.6 ± 1.45

Values are given as mean ± SEM. Statistically significant difference from control by Dunnett test (*$p < 0.05$). Abbreviations: RBC red blood cells, Hb haemoglobin, Ht hematocrite, WBC white blood cell, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration.

Table 2. Body, Brain and Relative Brain Weights of Rats in Control and Cypermethrin Treatment Groups ($n = 10$)

Parameters	Control	60 mg/kg	150 mg/kg	300 mg/kg
Initial Body Weight (g)	157.1 ± 24.2	120.0 ± 28.7	121.0 ± 15.9	126.3 ± 14.3
Final Body Weight (g)	180.0 ± 25.1	173.1 ± 21.4*	154.8 ± 13.7*	155.9 ± 11.3*
Weight of Brain (g)	1.69 ± 0.05	1.66 ± 0.03	1.49 ± 0.05	1.52 ± 0.09
Relative Brain Weight	0.008 ± 0.001	0.010 ± 0.001*	0.010 ± 0.001*	0.010 ± 0.002*

Values are given as mean ± SEM. Statistically significant difference from control by Dunnett test (*$p < 0.05$).
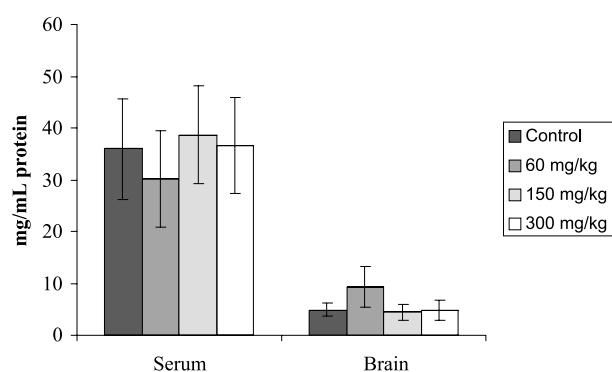
Fig. 1. Serum and Brain Total Protein Values of Rats in Both Control and All Doses of Treatment Groups
Values are given as mean ± SEM. $n = 10$ for each group.
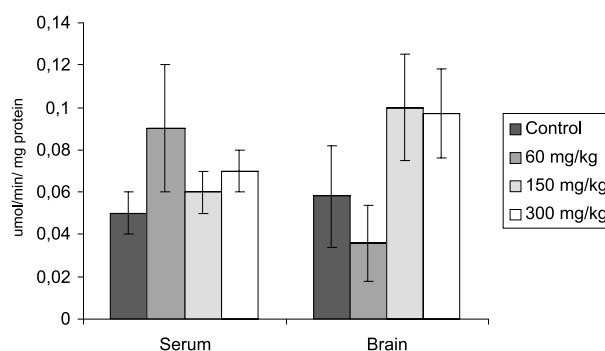
Fig. 2. Serum ChE ($\times 10^{-1}$) and Brain AChE Enzyme Activities of Rats in Both Control and All Doses of Treatment Groups
Values are given as mean ± SEM. $n = 10$ for each group.

tween control and exposure groups. However, brain AChE activities were increased in rats treated with 150 and 300 mg/kg cypermethrin, although these results were not statistically significant.

Histopathological Observations

Histopathological changes were observed in the brain tissues of rats in all treatment groups compared to control group. In contrast to the histological examination of the brain tissue of controls (Fig. 3A),
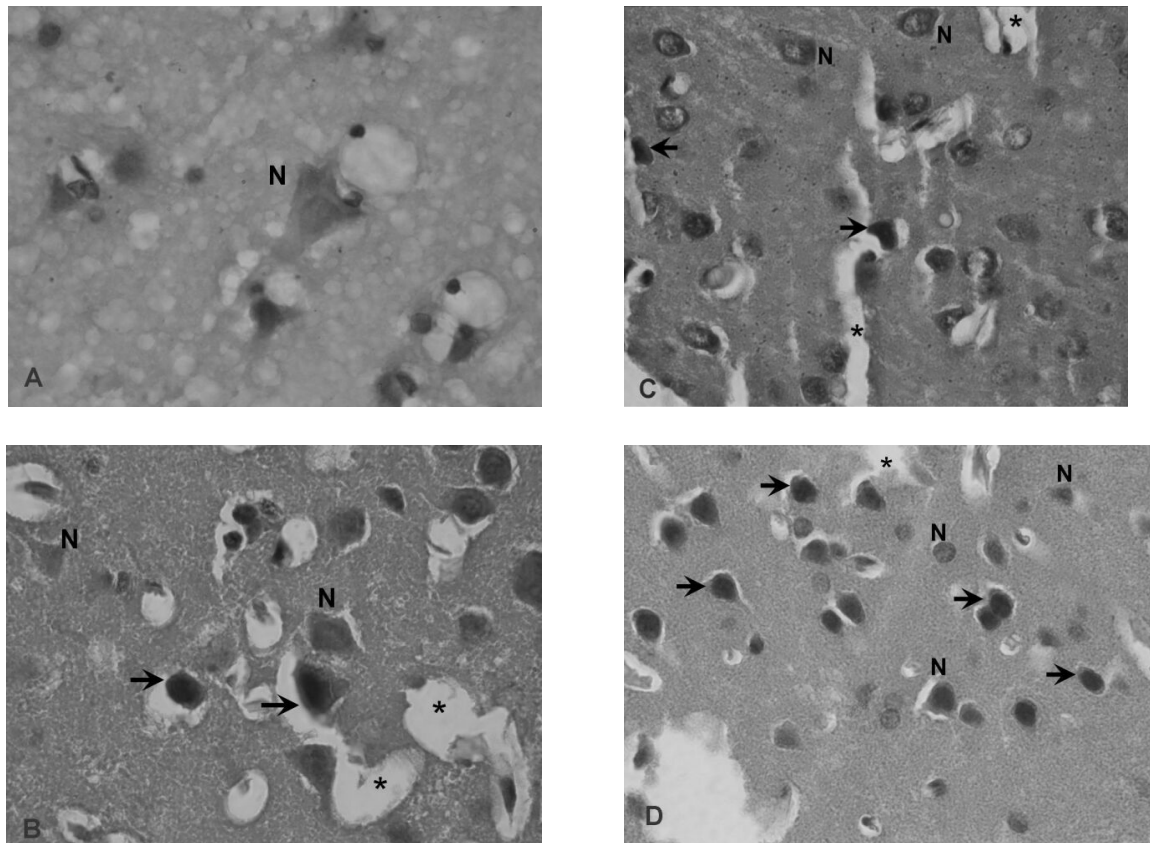
Fig. 3. Micrographs of the Brain Tissue Showing a Normal Histological Structure in Control Group (A), Mild to Severe Deformation Areas (*) due to Ischemia and Pyknosis (Arrow) of the Cytoplasm of the Neurons (N) in the Brain Tissues of Rats Treated with 60 mg/kg (B), 150 mg/kg (B), and 300 mg/kg (C) Cypermethrin
H&E Stain; Original magnification, × 100.

severe deformations due to ischemia and pyknosis of the cytoplasm of the neurons were observed in the brain of all cypermethrin treated groups (Fig. 3B–3D). In addition, the histopathological changes in the brain tissue have shown a dose–dependent increase in cypermethrin treated groups.

DISCUSSION

In this study, there were no significant changes body weight gains of rats of all cypermethrin treated groups in comparison with control animals. Aldana et al.[6] reported that a statistically significant decrease (11%) in body weight, in i.p. administration of 300 mg/kg cypermethrin to male rats for 7 days. However, some authors reported that deltamethrin, a synthetic pyrethroid, had no significant effect on body weight.[26,27] In this regard, our findings are consistent with their results. In the current study, however, cypermethrin had no significant effect in total

proteins of serum and brain. Shaker et al.[27] found that serum total proteins of deltamethrin treated rabbits decreased in a dose dependent trend. Interestingly, Aziz et al.[26] showed that the activity of AChE in hippocampal region significantly increased in 1 mg/kg deltamethrin-administered rats. Similarly, Husain et al.[17] reported that, in their studies with deltamethrin (orally administration of 7 mg/kg wt cypermethrin in corn oil for 15 days), a significant increase in the activity of AChE was observed in brain tissue. They also reported that AChE activity increased after 1 month of treatment with deltamethrin in rabbits. In our study, similar to deltamethrin, serum ChE and brain AChE activities were increased in rats treated with 150 and 300 mg/kg cypermethrin, but not statistically significant. Different doses, different solvents and different administration routes were used in the various studies. All of the parameters may change the results of the study. For example, the oral LD_{50} for cypermethrin in rats is 250 mg/kg in corn oil or

4123 mg/kg in water.[20,21] This wide variation in toxicity may reflect different mixtures of isomers in the materials tested. The ratio of cis/trans isomers affects the toxicity, the additives and impurities could also modify the effects.

The results of our study showed that cypermethrin caused a dose–dependent decrease in some haematological parameters of the rats such as RBC, Ht and thrombocyte values. Especially, high dose cypermethrin treatment in this experiment caused a significant decrease in Hb concentration. We think that the decrease in Hb value is due to an increase in the rate at which Hb is destroyed. Our results are in accordance with the results indicating a disruption of erythropoiesis or an increase in destruction of blood cells.[28] Furthermore, there was congestion in liver, which may indicate a decrease in the RBC values in cypermethrin treated rats (data not shown). The increase in WBC, lymphocyte and monocyte was noted cypermethrin treated rats compared to the control group. This result is consistent with the literature showing cypermethrin have not an immune-suppressive effect on rats, although more parameters than done here have to be examined to talk about this subject.[29,30] While Hb value decreased significantly in rats treated with high dose cypermethrin, MCH values showed a significant dose–dependent decrease in medium and high dose cypermethrin treatment. As a result, it was apparent that cypermethrin caused the negative alteration on some haematological parameters. Indeed, alterations in the haematological parameters due to pyrethroids were extensively investigated, but very little attention has been paid to morphological changes induced by cypermethrin.

Histologically, the evaluation of brain specimens from rats exposed to cypermethrin in different dosages disclosed the presence of different histopathological changes in rats. Previously, Latuszynska *et al*.[31,32] reported some histopathological changes in various areas of the brain as well as increased density of the cytoplasm in neurocytes as a result of dermal application of chlorpyrifos and cypermethrin in the rats. The changes observed were most striking in the cells of cornu ammonis (CA) 1 and CA 3 hippocampus layers, the hypothalamus and the stratum granulosum in area dentate.[31] As reported by other authors, focal pyknosis of the cytoplasm was observed in the cortex cerebri and the cerebellum.[31,32] Luty *et al*.[33] also reported that changes concerning the Purkinje cells in the cerebellum, concentrations of the cytoplasm of single pyramidal cells of CA 3

hippocampus layer, and a focal pyknosis of the neurocytes of nuclei lateralis hypotahalami and the cerebral cortex in rats exposed to 250 mg/kg alpha-cypermethrin. In this study, we also demonstrated development of ischemia and pyknosis of the cytoplasm of the neurons in the brain tissues of rats exposed with cypermethrin. To the best of our knowledge, such a dramatic structural alteration due to cypermethrin administration has not been reported previously. Based on our results and literature data, there is no doubt that cypermethrin exerts a neuro-toxic effect which is manifested by an increasing of ChE activity and the impairment of neural conductivity in the central and peripheral nervous system. Moreover, various researchers have documented behavioral effects of cypermethrin in the literature.[34–36] Nevertheless, we believe that the studies addressing the comparison of histopathological and behavioral effects would be very helpful in the evaluation of pyrethroid insecticides.

In conclusion, the overall results of this study clearly demonstrate that oral administration of the cypermethrin leads slight histopathological changes in the brain, as well as various blood parameters in rats. Low toxicity of pyrethroid insecticides for mammals is explained by their rapid biotransformation and discharge in urine. Although the fact that an extrapolation from rat to humans should not be done, one conclude that cypermethrin usage might cause hazardous effects in various levels to non-target organisms, including human being. In clinical practice, it should be considered in the differential diagnosis of ischemia of the brain tissue presenting as cerebral stroke signs and symptoms.

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